techniques. A description of the construction of the expression vector and CHO cell expression system can be found in U.S.S.N. 09/441,654, filed November 12, 1999, entitled "Method of Producing Glycosylated Bikunin," by Inventor Sam Chan. Briefly, the expression vector pBC-BK was constructed by cloning bikunin cDNA immediately downstream of the cytomegalovirus immediate early promoter and upstream of the polyadenylation signal sequence. The expression vector pBC-BK consists of a transcription unit for bikunin, dihydrofolate reductase, and ampicillin resistance. Bikunin cDNA was released from the cloning vector by restriction enzymes, blunt-ended, and ligated to linearized pBC. The linearization of pBC was done by a single restriction enzyme digestion. The orientation of bikunin cDNA was confirmed by sequencing.

B1

3. Please replace the paragraph at page 71, line 29 to page 72, line 12 with the following paragraph:

About 1 x 10^6 CHO (Chinese hamster ovary) cells were transfected with 10 μg of pBC-BK using Lipofectin reagents (Life Technology, Bethesda, Maryland) according to manufacturer's instructions. The cells were then selected in the presence of 50 nM methotrexate and grown in DME/F12 media deficient in thymidine and hypoxanthine plus 5% dialyzed fetal bovine serum. Cell populations were screened for bikunin production with a chromogenic assay. Briefly, bikunin standards or culture fluid was serially diluted and incubated with an equal volume of kallikrein at 37° C for 30 minutes after which a chromogenic substrate, N-benzoyl-Pro-Phe-Arg-pNA, was added. The reaction was incubated for 15 minutes before the addition of 50% acetic acid. The amount of p-nitroanilide released was measured at 405 nM. The high producing populations were further selected in media containing increasing concentrations of methotrexate (100 to 400 nM methotrexate) and screened for the production of bikunin. Limiting dilution cloning was then applied to derive clones with high and stable productivity. The cloning was done in the absence of methotrexate using standard tissue culture techniques by depositing 1 cell/well in 96-well plates. A clone designated FD3-1 was chosen for productivity evaluation in a bioreactor and was deposited on November 12, 1999 with the American Type Culture Collection (ATCC), Rockville, MD, and was assigned accession number PTA-940.

B2

4. Please replace the paragraph at page 75, lines 2-17 with the following paragraph:

Overall yield of bikunin was about 30% with a purity of 95%. Mass spectroscopy data also suggested that in addition to full length Bikunin (1-170) molecules, species lacking three (G-S-K) and four (L-G-S-K) (SEQ ID NO:106) amino acids from the carboxy end of Bikunin (1-170) were present in the pure protein pool. The material produced was shown to be stable to degradation when exposed to 72-hours incubation at ambient temperature or at 37°C, neutral pH. N-terminal sequencing, gel electrophoresis, immunoblotting, and amino acid

B3

analysis indicated that the bikunin was substantially pure (no other sequences were detected). An additional reverse phase chromatography step revealed that the CHO-derived purified bikunin was still able to be fractionated into several species (Figure 30A). CHO bikunin (8.5 mg) was adjusted to pH 2.5 with trifluoroacetic acid (TFA, 0.1% final concentration) and subjected to chromatography on a C18 reverse-phase column (Vydac, 2.5 x 25 cm) equilibrated in 17.5% acetonitrile and 0.1% TFA at a flow rate of 2 ml/min. CHO bikunin was eluted with a linear gradient of 17.5-40% acetonitrile in 0.1% TFA over 60 min. Figure 30B shows the silver stained SDS-PAGE profile of these fractions (lane between 54 and 55 represents molecular size markers).

5. Please insert the new sequence listing, which the applicants submitted on September 9, 2002, into the written description in place of the current sequence listing.

In the Claims:

1. Please cancel claims 12-14 without prejudice or disclaimer of the subject matter claimed therein.

2. Claims 1, 15, 16, 17, and 18 are amended.

The pending claims are shown in Appendix B. The rewritten claims, with markings to show the changes made, are shown in Appendix C.